International Journal of Bio-Technology
and Research (IJBTR)
ISSN (P): 2249-6858; ISSN (E): 2249-796X
Vol. 5, Issue 4, Aug 2015, 11-18

EFFECT OF PHOSPHORUS SUPPLY ON YIELD AND NODULE FUNCTIONING OF *C. ARIETINUM* L. GROWING UNDER SALINITY

H. SADJI-AIT KACI[1], A. CHAKER HADDADJ[2] & F. AID[3]

[1,2]Equipe Biologie Des Sols, Laboratoire De Biologie Et Physiologie Des Organismes, Faculté Des Sciences Biologiques, Université Des Sciences Et De La Technologie Houari Boumediene (USTHB), Alger, Algérie

[3]Equipe Physiologie Végétale, Laboratoire De Biologie Et Physiologie Des Organismes, Faculté Des Sciences Biologiques, Université Des Sciences Et De La Technologie Houari Boumediene (USTHB), Alger, Algérie

ABSTRACT

Crop yields are primarily limited under salinity conditions in Mediterranean region. This study was conducted to determine the effects of two doses of phosphorus fertilization (90kg P_2O_5 ha^{-1}, 200kg P_2O_5 ha^{-1}) in saline soil on improvement of crop productivity and salinity tolerance of chickpea cv Flip 74-92C.

Salinity may not effect plant growth but reduced nodulation and crop production. Indeed, salt stress expressed greater adverse effects on nodule traits consequently affected nodule functioning. However, the mixture of fertilizer to salinity (90kg P_2O_5 ha^{-1}X NaCl) improved significantly grain yield (about 30%).

This combination reduced significantly pro line content, and protected membranes against peroxydation. Indeed, the low P level mixed to salinity induced similar responses and sometimes better to plants control. This approach might attenuate and/or suppressed the adverse effects of salinity, increase the uptake of nutrients from the soil and improve salinity tolerance.

KEYWORDS: P Supply, Chickpea, Biomass, Nodulation, Nodule Traits, Salt Stress, Tolerance

INTRODUCTION

Cicer arietinum L. (chickpea) is a symbiotic species cultivated worldwide. Its grain is considered an important source of proteins (25.3-28.9%) for human food (Hulse et al., 1991). However, several environmental factors, such as salinity, low soil nitrogen (N) or phosphorus (P) levels are important constraints worldwide for leguminous crops (Graham & Vance, 2003).

Saline soils occupy 3.2 million hectares in Algeria (Le Houérou, 1993) and more than 800 million ha throughout the world (Fao, 2008). Salinity disrupts physical and chemical proprieties of the soils, and reduces water permeability (Agassi et al., 1981). Ions (Na^+ and Cl^-) reduce the activity of microorganisms as a result of low water availability and ion toxicity (Marschner, 2012) which influences negatively the plants growth and exerts a depressive effect on nodule establishment and functioning (Tejera et al., 2006; L'Taief et al., 2007). In addition, salinity can alter nutrient uptake through antagonistic effects with essential nutrients (Shibli, 1993).

Phosphorus (P) is also factor limiting of deficiency efficient symbiosis with Rhizobia and eventually the number of seeds per plant (Singh et al., 2011). The deficiency of P might limit N_2 fixation through its effects on growth of Rhizobia, nodule formation, nodule functioning and host-plant growth (Tang et al., 2001). In Algeria, chickpea crop

depends on efficient application of 200kg P_2O_5 ha^{-1} of superphosphate fertilizer (TSP). Exploitation of saline soil is possible by selection of tolerant chickpea lines (Flowers et al., 2010) and/or enhancing saline soil fertility.

The aims of the present study were to assess the role of P in symbiotic N_2 fixation efficiency and the productivity of chickpea growing under salt stress.

MATERIAL AND METHODS

The field experiment was conducted at the Experimental Farm of ITGC (Institut Technique des Grandes Cultures) Algiers, Algeria, using chickpea cv Flip 84-92C sown in April and harvested in August. The experiments were carried out following a bi-factorial statistic pattern following a randomized block design. The first factor was phosphorus application with two levels. The second factor was NaCl application with two concentrations.

The soil analysis of experimental site revealed that soil is silt-clay with pH 7.2, organic matter 1.88%, phosphorus available 15.6 mg kg^{-1}, electric conductivity 0.18 dS cm^{-1} and the rate C/N $\geq$ 7 for good biological activity. The experimental design is a randomized complete block, where plots represent all treatments. Seedlings of chickpea cultivar Flip 84-92C selected by ICARDA were planted in six rows with spacing of 1m between rows and 10 cm between plants. Row distance was 1m and plot size was 15 m^2. Plant density was 40 plants m^{-2} and 400 plants $plot^{-1}$.

Prior a nodulation by indigenous M. ciceri strains, the plants were irrigated with six different nutritive solutions. Control was irrigated with water (C); salt-treated plants received 150mM NaCl (S). Plants irrigated with 90kg P_2O_5 ha^{-1} (P1) and then watered with 200kg P_2O_5 ha^{-1} (P2). Plants irrigated with a saline solution combined to 90kg P_2O_5 ha^{-1}(SP1), or combined to 200kg P_2O_5 ha^{-1} (SP2). All solutions were prepared in distilled water. Field was raised for a period of five months. Samples were taken each time from each plot and the average values for all parameters represent the means of three replicates.

Three plants are collected and dried at 70C°. Dry weight for each part was measured including shoot dry weight (SDW), root dry weight (RDW), Root/Shoot ratio and total dry weight.

Relative Water Content (RWC) of leaves was estimated using the following equation based on SFW (shoot-fresh weight) and TW (turgid weight):

RWC (%) = 100 X [(SFW – SDW) / (TW – SDW)]

C arietinum L.-M ciceri symbiosis establishment was estimated by nodule number and nodule dry weight (NDW). Total protein was determined by Bradford (1976). Proline content estimated by Troll & Lindsley (1955) method.

MDA in the leaves was analyzed following Carmak and Horst (1991). This method is based on the reaction with thiobarbituric acid. Fresh leaves (1.0 g) were ground properly in 20 ml of 0.1% tri-chloroacetic acid solution and centrifuged for 10 min at 12000 g. One ml of the supernatant was reacted with 4 ml of 20% TCA solution comprising 0.5% thiobarbituric acid and then it was heated for 30 min., at 95^{o}C in a water bath and then immediately cooled on ice. After centrifugation for 10 min., at 12000 g, the absorbance of the supernatant was read at 532 and 600 nm. The contents of MDA were worked out using the extinction coefficient of 155/ (mM/cm) using the formula: **MDA level = Δ (A 532nm-A 600nm)/1.56×105**

RESULTS

Chickpea responded to phosphorus application, but this response varied according to of treatments. No significant differences on dry matter partitioning (RDW) between plants organs. However, RDW of P1 (90 kg P_2O_5 ha^{-1}) was significantly higher than those of other cases (Table 1). Indeed, P2 and SP2 treatments had enhanced nodular biomass (NDW), which reached 9,2mg NDW^{-1} while in the control and saline nodules, the NDW varied between 4.6 and 5.4mg NDW^{-1}.

Low P level increased the root to shoot weight (RSR) about 75%, whereas it was similar for the remaining treatments (Table 1). This result indicated that without salinity, the low P level might stimulate the root growth. In contrast, under salinity conditions, the P fertilizer reduced the root development, probably due to the less salt sensibility observed in growth parameters.

Salinity x P mixture had not influence on leaf relative water content (RWC). SP1 plants showed RWC similar to control plants. It noteworthy, that RWC reduced but not significantly (30%) in SP2 plants and 16-21% in P1, P2 and saline plants compared to the control (Figure 1).

Salinity alone or mixed to (200kg P_2O_5 ha^{-1}) decreased nodule protein content (PTC) about 36% and 52% of that of control nodule. Whilst in SP1nodule, protein content increased about 52% than of to saline nodule. Without salt stress, nodule PTC remained unchanged with both P fertilizer applications (Figure 2A).

Nodule pro line content (PLC) significantly increased ($p \leq 0.05$) under salinity. In contrast, nodule of (salinity x low P level) combination accumulated pro line about 3-fold less than in saline and non-saline treatments (Figure 2B). Salt stress and low P level separately; the nodules produced the maximum of malonyldialdehyde (MDA) than of control nodules. However, the salinity mixed to low P supply reduced significantly the MDA content in nodule less than control, P1 and P2 treatments (Figure 2C).

DISCUSSIONS

Statistical analysis revealed significant differences between treatments among parameters. This indicated that an adequate amount of phosphorus fertilizer had influence on the response of plants. However, dry biomass remained unchanged with or without salinity (Table 1).

Results which signified that salinity had not inhibited the plants growth of cv Flip 74-92C. This response is disagree with those found by works which reported reduction of shoot and root biomass of plants under salt stress (Panattagul & Thitisaksakul, 2008). Nevertheless, high P level (200kg P_2O_5 ha^{-1}) with or without salinity increased significantly the nodule dry weight which presumably implied maintenance of total plant biomass. Root and shoot ratio (RSR) increased under P fertilizer but decreased under salinity conditions. This response was not in accordance with those found by (Aydi, 2008) who detected pronounced salt effect on shoot growth than root. However, a great RSR was obtained when low P level was applied. The author has associated the increase in root growth in order to decrease of osmotic stress by increasing of water influx (Aydi, 2008). Increased root growth may possibly be resulted from reallocation of photo synthetates into the root, instead of the shoot causing a reduction in shoot growth (Panattagul &Thitisaksakul, 2008). The effect of salinity on nodulation has been attributed to decrease in rhizobial colonization and shrinkage and lack of root hair formation (Swaraj & Bishnoi, 1999). Nevertheless, the addition of P fertilizer to saline-plants allowed a maximum of nodulation like

effect of P application alone had more than control plants. This result probably reflects the perfect protection of earlier establishment of new nodules against the accumulation of ions Na^+ and Cl^- (Krouma, 2009) (Table. 1).

The ions at high concentrations in saline soil (e.g. Na^+, Cl^-) are taken up by high rates, which may lead to the suppression of uptake of other essential ions and their transportation to the plant (Mer *et al.,* 2000). Nevertheless, the (P fertilizer X salinity) combination might dilute NaCl concentration in the soil and compromised the excessive influx of Na^+ into the plant which is correlated to the tolerance of plant to salt stress and increased the plant crop (Munns *et al.,* 2006).

Salinity, P1, P2 and SP2 treatments reduced the RWC (Figure 1). Despite, this reduction was not significant, this result indicated that salinity induced osmotic stress and had exerted a slight negative effect on plants. Reduce of RWC may be the consequence of low water uptake and flow rates within plants, or high water loss rates (Aydi, 2008). Interestingly (salinity X 90kg of P) had maintained the RWC similar to control plants. Response which suggest that combination allowed a tolerance of tissue to dehydratation (Iannucci *et al.,* 2002), by accumulation of osmolytes which are direct products of photosynthesis (Monneveux & belhassen, 1996). This osmotic adjustment helps to maintain shoot functioning (Aydi, 2008).

Nodule proteins soluble and proline content were differed significantly under salinity with or without P application. Protein concentration decreased about 50% under salinity whereas it increased about 50% when the plants were exposed to salt X low P level. Indeed, salinity accumulated a maximum of proline concentration whereas SP1 nodule reduced about 50% nodular proline. Trotel- Aziz *et al.,* (2000) have proposed that proline accumulated under stress conditions is rapidly consumed when stress conditions were alleviated. In this regard, we suggest that low P level combined to NaCl had essential rule in the shrinking e response of plant to salt stress (Figure 2A, B).

Malonydialdehyde (MDA) was significantly different for all combinations. MDA due to lipid peroxydation of cell membrane is often used as an indicator of salt and oxidative damages (Mandahania *et al.,* 2006). MDA concentration of saline nodules was highest than to control nodule. P application alone induced the similar MDA content than control nodule. Massive reactive oxygen species (ROS) generated during stress conditions may have the deleterious effects in plants tissue by the peroxydation reaction of membrane lipids (Ratnayaka *et al.,* 2003). However, lowest MDA concentration was obtained in (low P fertilizer X salinity) nodule (Figure 2C).This result indicated that P supply might protected the membranes against the adverse effects of salinity.

CONCLUSIONS

From the foregoing results, it may be conclude that the application of phosphorus fertilizer particularly low level (90kg P_2O_5 ha^{-1}) in saline-soil induced changes in condition field of chickpea cv. Flip 84-92C and may allow a tolerance of plants to salinity. These changes revealed by enhance crop yield and beneficial effect on symbiotic trait of nodules essentially by decline of stress indicators.

Adding P to saline soil might correct condition field by creating an ionic and osmotic balance in the rhizosphere, which may have attenuated and/or suppressed the drastic effects of salinity. Adding of (90kg $P_2O_5 \cdot ha^{-1}$) to saline soil could be an alternative for reducing the negative effects of salinity and might improve salinity tolerance.

REFERENCES

1. Agassi, M., Shainberg I., & Morni J. (1981). Effect of electrolyte concentration and soil sodicity on filtation rate and crust formation. Soil Sci. Am. J. 45: 848-851.
2. Aydi, S.S., Aydi, S., Gonzalez, E., & Abdelly C. (2008). Osmotic stress affects water relations, growth and nitrogen fixation in Phaseolus vulgaris plants. Acta Physiol. Plant. Doi: 10.1007/s11738-008-0141-y.
3. Bradford, M.M. (1976). A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytica Biochimisry. 72:248-254.
4. Carmak, I., & Horst, J. H. (1991). Effects of aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (Glycine max). Physiol. Plant. 83:463-468.
5. Fao. (2008). Land and Plant Nutrition Management Services. http://www.fao.org/agb/agl/agll/spush/
6. Flowers, T.J, Gaur, P.M., Gorda, C.L., Krichnamurthy, L., Samineni, S., Siddique, K.H.M., Turner, N.C., Vadez, V., Vashney, R. K., & Colmer, T.D. (2010). Salt Sensivity in chickpea. Review. Plant. Cell. Env. 1365: 3040-3060.
7. Graham, P.H., & Vance, C.P. (2003). Legumes: Importance and constraints to greater use. Plant Physiol. 3, 872–877.
8. Hulse, J.H. (1991). Nature, composition and utilization of grain legumes. In Uses of Tropical Legumes. Proceeding of Consulting Meeting, 27-30 March 1989. Édition Patencheru. ICRISAT Center, India. 502-524.
9. Iannucci, A., Mario, R., Lucia, A., Natale, D.F., & Pasquale, M. (2002).Water deficit effects on osmotic ajustement ans solutes accumulation in leaves of annual clovers. Eur. J. Agron. 16:111-122.
10. Krouma A. (2009). Physiological and nutritional response of chickpea (Cicer arietinum L.) to salinity. Turk J Agric For. 33:503-512.
11. L'Taief, B., Sifi, B., Zaman-Allah, M., Devon, J.J., &Lachaal, M. (2007). Effect of salinity on root – nodule conductance to the oxygen diffusion in the Cicer arietinum – Mesorhizobium ciceri symbiosis. J. Plant. Physiol. 164. 1028-1036.
12. Le Houérou, H.N. (1993). Salt-tolerance plants for the arid region of the Mediterranean isoclimatic zone. In: H. Leith, A. Al Massoom (eds), towards the rational use of high salinity tolerant plants. 1: 403-422.
13. Marschner, P. (2012). Marschner's mineral nutrition of higher plants. Academic Press, San Diego, CA.
14. Mer, R.K., Prajth, P.K., Pandya, D.H., Pndey, A.N. (2000). Effect of salt on germination of seeds and growth young plants of Hordeum Vulgare, Tiritikum Aestivum, Cicer Arietinum and Brassica Juncea.J. Gron. Crop. Sci., 185: 209-217.
15. Monneveux, P., & Belhassen, E. (1996).Thediversity of drought adaptation in the wide. In:Belhassen (ed) Drought Tolerance in Higher Plants, Genetical, Physiological and Molecular biological Analysis. Kluwer Academic Publishers, Dordrecht.
16. Mudgal, V., Madaan, N., Mudgal, A., Singh, A., & Kumar, P. (2010). Comparative Study of the Effects of

Salinity on Plant Growth, Nodulation, and Legheamoglobin Content in Kabuli and Desi Cultivars of Cicer arietinum (L.). KBM Journal of Biology. 1:1-4

17. Munns, R., James, R.A., & Läuchli, A. (2006). Approaches to increasing the salt tolerance of wheat and other cereals. J. Exp. Bot. 57 (5): 1025-043.
18. Panattagul, W., & Thitisaksakul, M. (2008). Effect of salinity on growth and carbohydrate metabolism in three rice (Oryza sativa L.) cultivars differing in salinity tolerance. Indian J.Exp. Biol. (46): 736-742.
19. Ratnayaka, H.H., Molin, W.T., & Sterling, T.M. (2003). Physiological and antioxidant responses of cotton and spurred anoda under interference and mild drought. J. Exp. Bot., 54: 2293-2305.
20. Shibli, R.A. (1993). Influence of increased salinity on yield fruit quality and growth of hydroponic grown tomatoes. Mu' tah J. Res. Studies 8:153-165.
21. Singh, A., Baoule, A., Ahmed, H., Dikko, A., Aliyu, U., Sokoto, M., Alhassan, J., Musa, M., & Haliru, B. (2011). Influence of phosphorus on the performance of cowpea (Vigna unguiculata (L) Walp.) varieties in the Sudan savanna of Nigeria. Agricultural Sciences 2:313-317.
22. Tang, C., Hinsinger, P., Drevon, J.J., & Jaillard, B. (2001). Nodule Functioning and Enhances Proton Release in Roots of Mdicago truncatula L. Annals of Botany. 88: 131-138.
23. Tejera, N.A., Soussi, M., & Llunch, C. (2006). Physiological and nutritional indicators of tolerance to salinity in chickpea plants growing under symbiotic condition. Env. Exp. Bot. 58: 17-24.
24. Troll, W., & Lindsey, G. (1955). A photometric method for the determination of prolin. J. Biol. Biochem. 215: 655-660.

APPENDICES

Table 1: Effect of Phosphorus Fertilization (0, 90 and 200kg P_2O_5 ha^{-1}) on Dry Biomass of Plant Part: Shoot (SDW), Root (RDW), Nodules (NDW), Root/Shoot (RSR) of *C.arietinum* L. cv Flip 74-92C Exposed to Salinity (0 and 150mM NaCl). Mean Comparisons among Treatments are the Small Letters. Means in the Same Category followed by One Common Letter are not Significantly Different at Post HOC Test, $p \leq 0.05$

Test	Treatment	SDW (g)	RDW (g)	NDW (mg)	RSR	
C	0mM NaCl	3.13 ± 0.74^{a}	0.28 ± 0.13^{ac}	4.6 ± 1.82^{a}	0.11 ± 0.07^{a}	
P1	90kg $P_2O_5ha^{-1}$	2.66 ± 0.76^{a}	1.78 ± 1.27^{b}	5.4 ± 1.15^{a}	0.80 ± 0.75^{b}	
P2	200kg $P_2O_5ha^{-1}$	2.35 ± 1.22^{a}	0.21 ± 0.09^{a}	9.5 ± 1.59^{b}	0.10 ± 0.01^{a}	
		2.10 ± 0.25^{a}	0.16 ± 0.01^{a}	4.7 ± 1.33^{a}	0.08 ± 0.005^{a}	
S	150mMNaCl	2.61 ± 1.11^{a}	0.37 ± 0.07^{ac}	5.1 ± 2.26^{a}	0.17 ± 0.07^{ac}	
SP1	S+ 90kg$_2$$O_5ha^{-1}$	1.81 ± 0.31^{a}	0.16 ± 0.09^{a}	9.2 ± 2.71^{b}	0.08 ± 0.3^{a}	
SP2	S+200kg$_2$$O_5ha^{-1}$	2.66 ± 0.76^{a}	0.28 ± 0.13^{ac}	4.6 ± 1.82^{a}	0.11 ± 0.07^{a}	

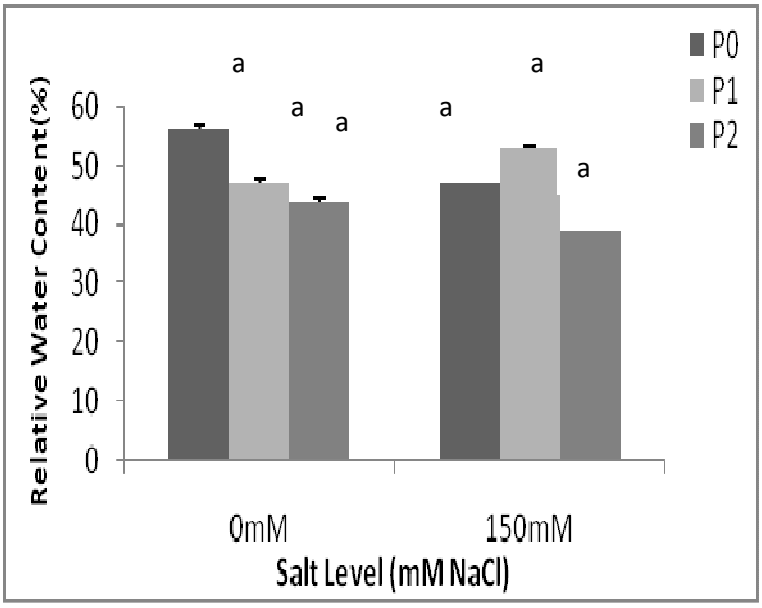
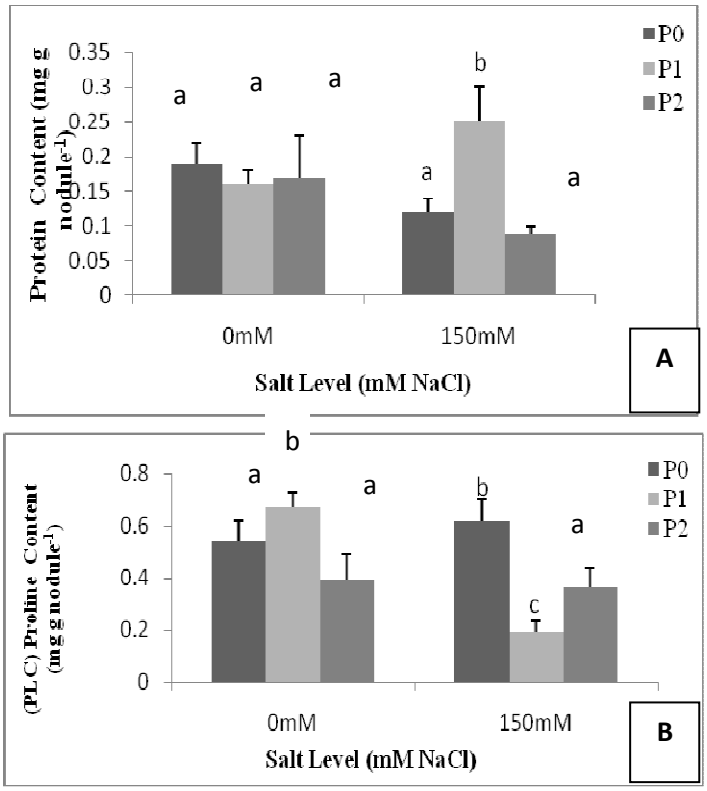


Figure 1: Effect of Phosphorus Fertilization (0, 90 and 200kg $P_2O_5ha^{-1}$) on Relative Water Content (RWC) of *C. arietinum* L. cv Flip 74-92C **Exposed to Salt Level (0 and 150mM NaCl). Values Represent the Average of Three Replicates. Mean Comparisons among Treatments are the Small Letters. Means Followed by One Common Letter are not Significantly Different at Post hoc Test, $p \leq 0.05$**



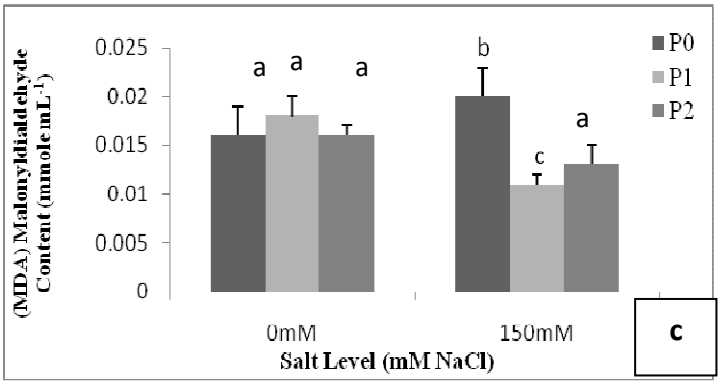


Figure 2: **Effect of Phosphorus Fertilization (0,90 and 200kg P_2O_5ha^{-1}) on (A) Protein Content, (B) Proline Content and (C) Malonyldialdehyde Content of Nodule of *C. arietinum* L. cv Flip** 74-92C **Exposed to Salt Level (0 and 150mM NaCl). The Plants were Harvested at 60DAS. Bars Represent ± SD of Three Replicates. Mean Comparisons among Treatments are the Small Letters. Means followed by One Common Letter are Not Significantly Different at Post hoc Test, $p \leq 0.05$**